Laboratory Investigation of Male Gonadal Function

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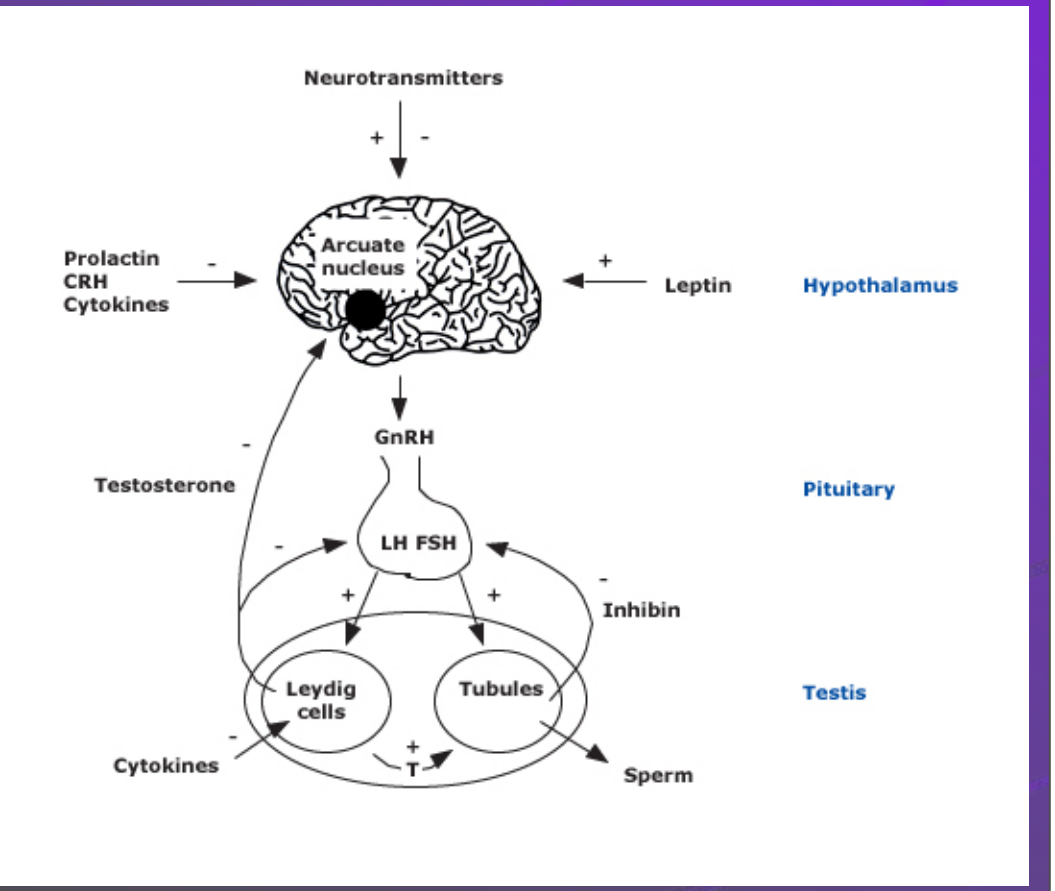
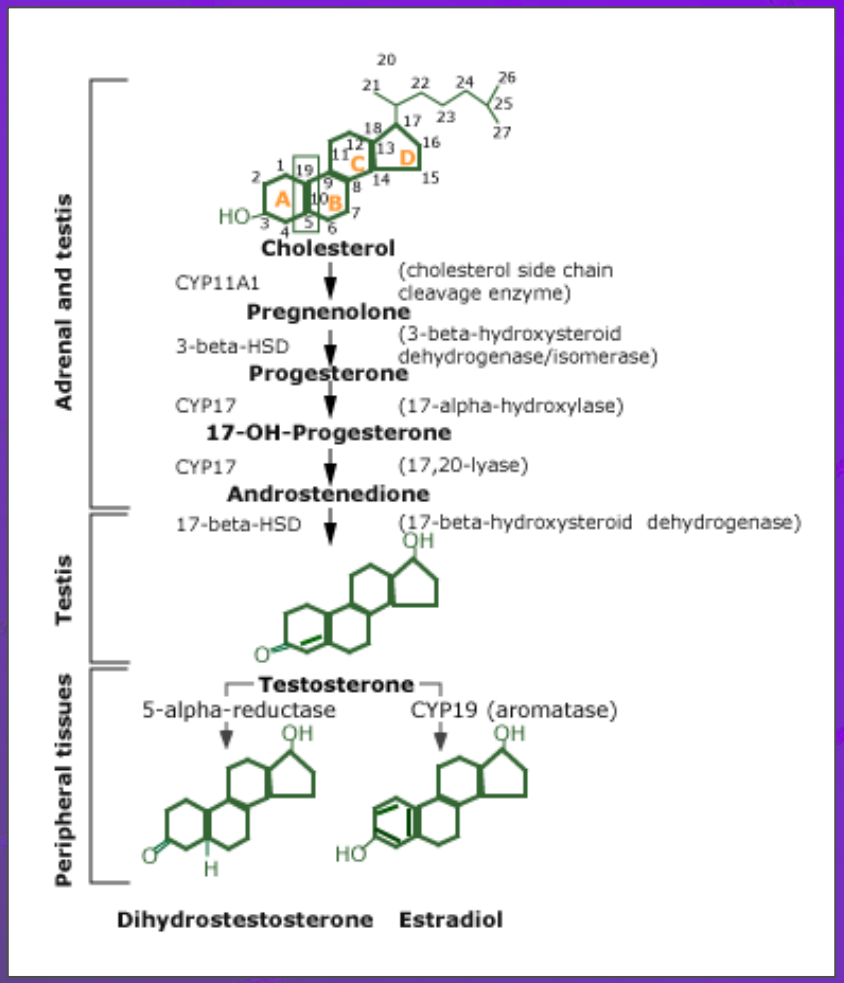
Figure 1. Hypothalamic-pituitary-testicular axis





Testosterone (T) measurement

- 60% of T is strongly bound to SHBG
- 38% of T is weakly bound to albumin
- 2% of T is free
- Free and albumin-bound fractions are “bioavailable”
- Free T assay is technically demanding
- Most labs calculate free or bioavailable T using [total T], [SHBG], [albumin]
- Online calculator at http://www.issam.ch/freetesuit.htm

Testosterone measurement (cntd)

- Free androgen index
  - (FAI) = [total T]/[SHBG] x 100
  - Use FAI only in females
  - Overestimates free T in males
- Diurnal variation in T with max values at 08h00; min values at 20h00

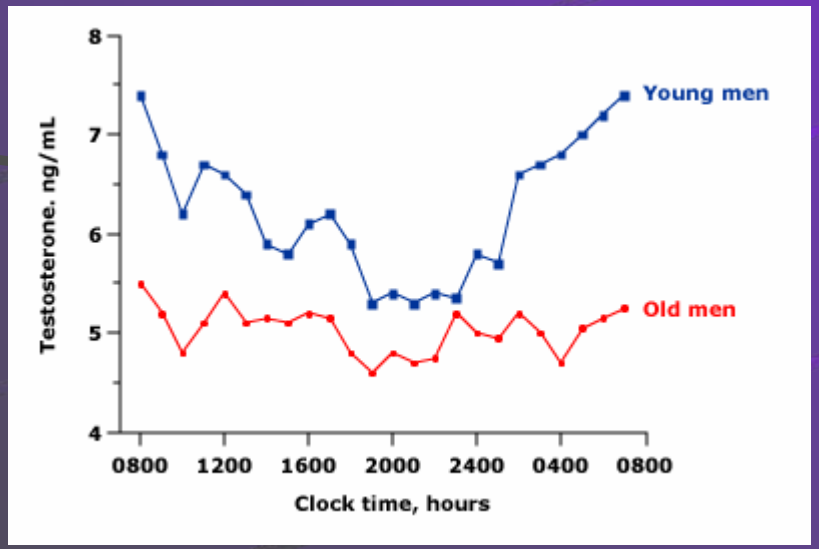


Table 1. Effects of T deficiency according to age at onset

Age	Effects of T deficiency
1st trimester in utero	Incomplete virilization of external genitalia
	Incomplete development of Wolffian ducts to form male internal genitalia
3rd trimester in utero	Micropenis
	Cryptorchidism
Pre-puberty	Incomplete pubertal maturation
	Eunuchoidal body habitus
	Poor muscle development and reduced peak bone mass
Post-puberty	Decreased energy, mood, libido
	Decreased sexual hair, Hct, muscle mass and strength, BMD
	Infertility

Table 2. Causes of abnormal SHBG

Increased SHBG	Decreased SHBG
Anorexia nervosa	Obesity
Hyperthyroidism	Hypothyroidism
Androgen deficiency	Androgens
Liver cirrhosis	Glucocorticoids
Excess oestrogens	Nephrotic syndrome
HIV infection	
Systemic illnesses	
Aging	

Male gonadal dysfunction

- Presenting complaints:
  - Delayed puberty (no onset by 14y)
  - Infertility
  - Erectile dysfunction
  - Gynaecomastia

- Laboratory tests
  - Prolactin
  - Testosterone, SHBG, cFT or bioavailable T
  - LH, FSH
  - TSH and FT4
  - Karyotype (selected pts)
  - Semen analysis
  - Cortisol, iron studies

Male gonadal dysfunction (cntd)

- 1° (hypergonadotrophic) hypogonadism
  - ↓T; ↑LH; ↑FSH; azoospermia
  - Testicular failure (see table 3.)
  - Selective germ cell dysfunction
    - ↔T; ↔LH; ↑FSH; azoospermia
  - Selective Leydig cell dysfunction
    - ↓T; ↑LH; ↔FSH; normozoospermia
- 2° (hypogonadotrophic) hypogonadism
  - ↓T; ↓/↔LH; ↓/↔FSH
  - Hypothalamic-pituitary pathology (see table 3.)
  - ↑ prolactin and systemic disease
  - Fertile eunuch sd is a form of IHH with ↓T; ↓/↔LH; ↔FSH; normozoospermia

Table 3. Causes of male hypogonadism
1° hypogonadism (↓T, ↑LH/FSH, impaired sperm production)
Genetic syndromes (Klinefelter's sd 47XXY, 45XO or mosaicism, streak gonads, myotonic dystrophy, Noonan's sd)
Cryptorchidism (untreated)
Vanishing testis syndrome
Testicular insults (trauma, torsion, infectious orchitis, radiation, chemoTx, drugs, autoimmune, HIV infection, haemochromatosis)
Androgen receptor defects (testicular feminization)
5-α-reductase deficiency
LH receptor defects
2° hypogonadism (↓T, ↓ or ↔ LH/FSH)
Genetic syndromes (Kallman's, Prader-Willi, idiopathic hypogonadotrophic hypogonadism [IHH], GnRH deficiency)
Hypothalamic-pituitary insults (trauma, insufficiency, tumours, infiltrates e.g., haemochromatosis, sarcoidosis; infections)
Systemic conditions (liver disease, ESRF, HIV infection, hyperprolactinaemia, excessive exercise, eating disorders, severe obesity)
Drugs (opiates, anabolic steroids, alcohol, marijuana)
Sperm transport disorders (↔T, ↔ LH/FSH, ↓ semen fructose)
Obstruction of epididymus/vas deferens (cystic fibrosis, congenital absence)

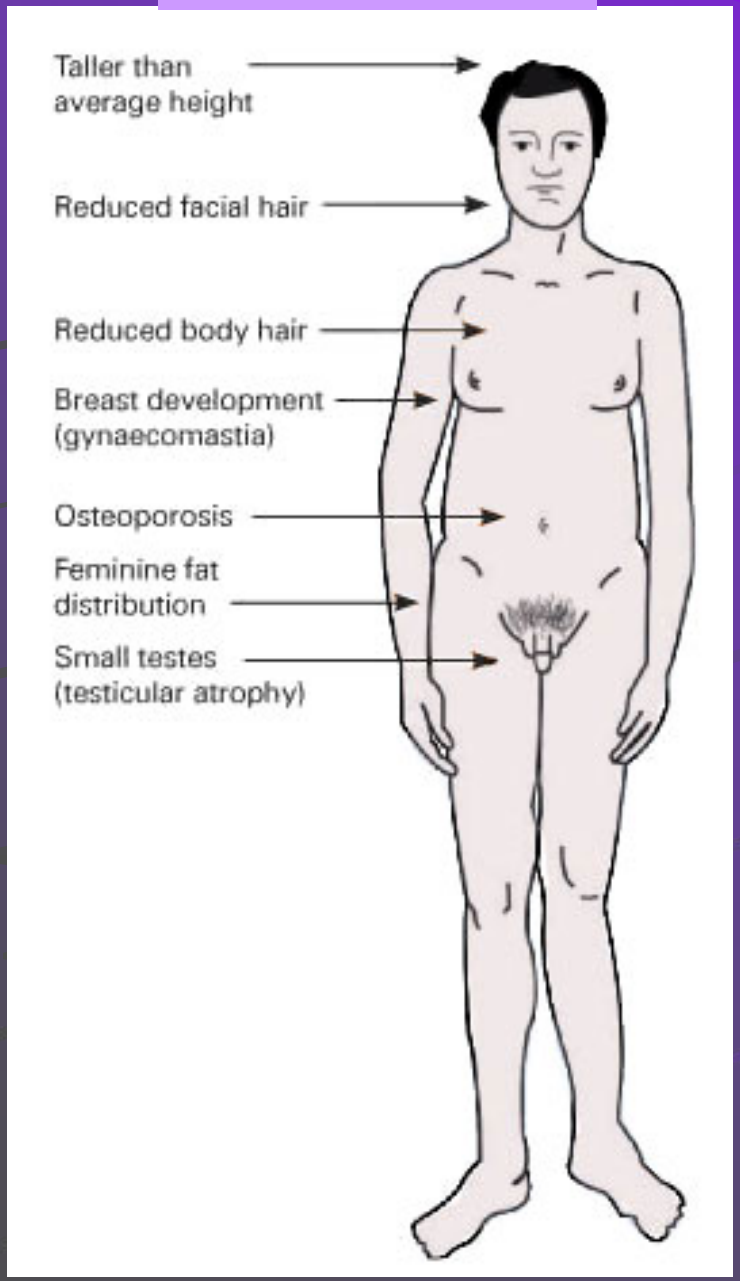
Case study - 1° hypogonadism

- An 18-year-old boy with tall stature, gynaecomastia, and behavioural problems
- On exam he was found to have small testes and sparce facial and pubic hair

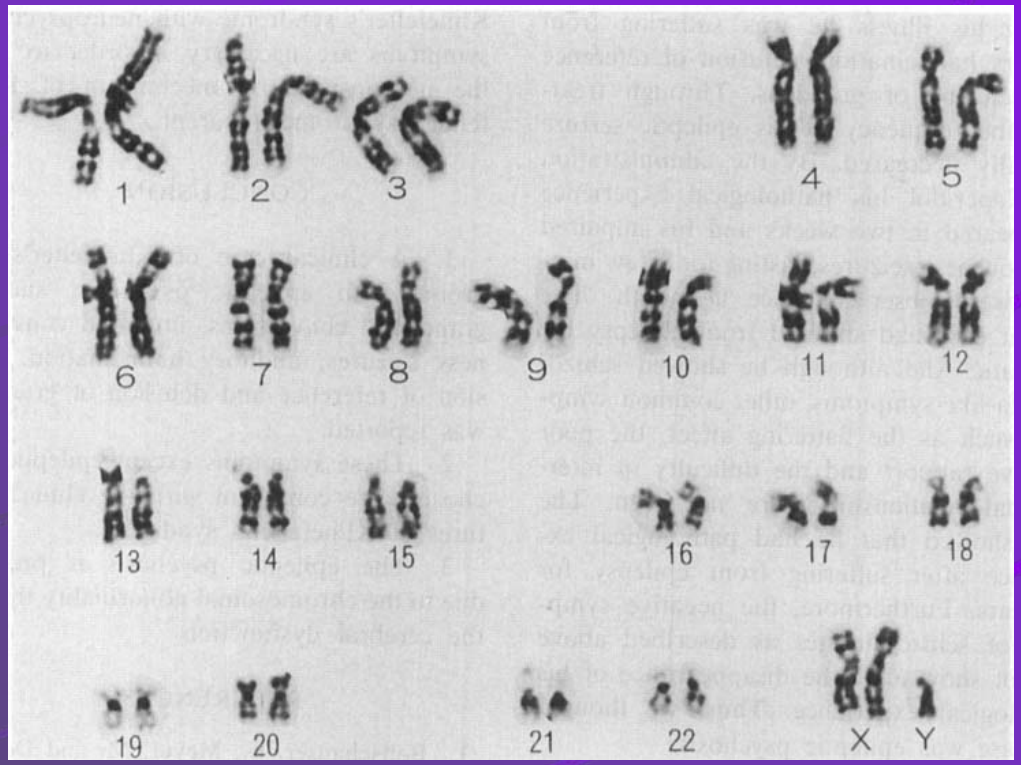
Plasma	
Karyotype	47XXY
LH (1.5-11.5)	34 U/L ↑
FSH (3-15)	76 U/L ↑
Testosterone (7-28)	6 nmol/L ↓

- Diagnosis: Klinefelter's sd (1:500)

Eunuchoid habitus



Chromosomal analysis revealing 47 XXY karyotype



Case study – complete androgen insensitivity syndrome

- 18-year-old *girl* with 1° amenorrhoea and sparse axillary/pubic hair, despite good breast development
- Further exam revealed short, blind-ending vagina, no uterus/ovaries and intra-abdominal testes

Plasma with female RR's		Male RR
Karyotype	XY	
E_2 (100-590)	73 nmol/L ↓	E_2 (<180)
LH (1.5-11.5)	27 U/L ↑	LH (1.5-9.0)
FSH (3-15)	1.7 ↓	FSH (1.5-9.0)
Testosterone (0.8-2.8)	25 nmol/L ↑	Testosterone (7-28)

- CAIS = testicular feminization

Case study – 5α reductase deficiency

- 15-year-old daughter of consanguineous parents presented with amenorrhoea, no breast development, sparse pubic/axillary hair
- Exam revealed clitoris-like phallus, pseudovaginal perineoscrotal hypospadia, no uterus/ovaries and inguinal testes

Plasma with female RR's		Male RR
Karyotype	XY	
LH (1.5-11.5)		LH (1.5-9.0)
FSH (3-15)		FSH (1.5-9.0)
Testosterone (0.8-2.8)	25 nmol/L ↑	Testosterone (7-28)
DHT (0.2-0.4)	0.2 nmol/L	DHT (0.1-0.6)
T:DHT	125	

Case study - 2° hypogonadism

- A 45-year-old man with tiredeness, decreased libido and decreased frequency of shaving
- He was diagnosed with diabetes mellitus 6 mnths earlier

Cortisol (140-550)	90 nmol/L ↓
Testosterone (7-28)	4 nmol/L ↓
LH (1.5-9.0)	3.0 U/L
FSH (1.5-9.0)	0.7 U/L ↓
TSH (0.15 – 3.5)	1.4 mU/L
FT4 (10 – 27)	8 pmol/L ↓
Prolactin (3-17)	15 μg/L
Iron (9-27)	24 μmol/L
Transferrin (2.1-4.3)	1.4 g/L ↓
% Trf saturation (10-55)	67 % ↑
Ferritin (30-300)	11 500 g/L ↑

- Panhypopituitarism + DM: consider haemochromatosis
- LFT's were abnormal and he was homozygous for C282Y

Other tests

- GnRH stimulation test
  - IV GnRH and measure LH/FSH at 0, 20, 60 min
  - LH increases 3-6x; FSH increases 20-50%
  - 1° hypogonadism: exaggerated responses
  - 2° hypogonadism: reduced responses
  - Infrequently used, since adds little value
- Clomiphene stimulation test
  - Oral Clomiphene for 5-7d; measure LH/FSH on day 0 and 7
  - LH increases 2x; FSH increases 20-50%
  - 2° hypogonadism: reduced responses
  - Helpful to distinguish idiopathic delayed puberty

Other tests (cntd)

- hCG stimulation test
  - IM hCG on day 0 and 2; measure T on day 0, 2 and 4
  - T should rise to above RR
  - 1° hypogonadism: reduced responses in Leydig cell hypofunction
  - 2° hypogonadism: exaggerated (3x increase) response
  - Cryptorchidism: no increase suggests absence of functioning testicular tissue; increase suggests intra-abdominal testes
  - 5α reductase deficiency (pre-pubertal): T:DHT >10
- Testicular biopsy for azoospermia with normal T, LH/FSH

Semen analysis

- Normal semen analysis:
  - >20 million sperm/mL
  - >40 million sperm/ejaculate
  - >50% motile; >25% have rapid forward progression; >50% have normal morphology
- Two normal analyses = normal
- Four abnormal analyses over mnths to diagnose abnormality
- <5 million sperm/ejaculate in 1°/ 2° hypogonadism
- 35 million sperm/ejaculate plus aberrant motility → sperm function abnormality or 1° hypogonadism

Gynaecomastia

- Proliferation of glandular tissue of male breast due to ↓ androgen:oestrogen ratio
- Pseudogynaecomastia in obese men
- Causes (see Table 4.)
- Laboratory tests
  - Testosterone, SHBG, LH, FSH, E_2, β hCG, prolactin
  - When indicated: tests of renal, liver, thyroid, adrenal, pituitary fx, karyotype

Table 4. Causes of gynaecomastia
Physiological
Neonatal, puberty, elderly
Decreased androgen synthesis or action
Hypogonadism
Increased oestrogen
Chronic liver disease **End-stage renal failure** **Hyperthyroidism** **Tumours**
Drugs
Oestrogens or binding to E_2-receptor (digoxin, cannabis, griseofulvin)
Anti-androgens (spironolactone, cimetidine)

Erectile dysfunction

- Causes of erectile dysfunction (see Table 5.)

- Laboratory tests for erectile dysfunction
  - Electrolytes, creatinine
  - Fasting glucose and lipogram
  - Testosterone, SHBG, LH
  - Prolactin

Table 5. Causes of erectile dysfunction
Neurogenic
Spinal cord injury, diabetic neuropathy
Psychogenic
Stress, performance anxiety, depression
Vasculogenic
DM, HT, atherosclerosis, Peyronie disease, trauma
Drugs
Antihypertensives, antidepressants, anti-androgens, alcohol abuse, cigarette-smoking
Endocrine
Hyperprolactinaemia, thyroid disease, hypogonadism
Systemic disease
DM, CRF, CVD, liver disease